Remarks

Status of the Claims

The claims have been amended, support for which can be found throughout the specification and in the claims as originally filed. Claim 36 has been canceled without prejudice or disclaimer of the subject matter contained therein. The amendments to the claims do not add prohibited new matter.

Information Disclosure Statement

The Office Action on page 2 objected Applicants previous submission of a certified translation without resubmitting the Information Disclosure Statement. The Office Action indicated that the previously submitted documents have been placed in the file. Accordingly, Applicants hereby re-submit with this response the Information Disclosure Statement and the accompanying Form SB-08.

Drawings

The Office Action on page 2 tentatively objected to Figure 5 for the presence of non-corresponding sequence identifiers. Applicants herein resubmit Replacement Drawings.

Sequence Compliance

The Office Action on page 3 objected to the sequence listing for failing to list sequences presented in a drawing. Applicants submit with this paper an updated Sequence Listing, as well as a replacement copy of Figure 5, to address the basis of this objection.

Claim Objections

The Office Action on page 3 objected to claims 23 and 24 for informalities in the claim language. Applicants have amended the claims as suggested by the Examiner.

Rejection under 35 USC § 112, second paragraph

Claims 23-36 were rejected on page 3 of the Office Action as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

The Office Action alleged that the metes and bound of the claims are unclear. Without acquiescing to the merits of the rejection, Applicants have amended the claims. Regarding the term "multiplex PCR", Applicants agree with the Examiner this term usually implies multiple pairs of primers amplifying multiple different fragments of DNA. However, according to the present invention, "multiplex PCR" means that several different fragments are amplified with one single pair of primers, each amplified fragment corresponding to a specific chromosomal rearrangement. The reason why several different fragments are amplified with only pair of primers is that a biological sample as recited in the claims comprises cells in which different V-J rearrangements have occurred. This is explained in the specification, at least at page 17, lines 10-23, and illustrated throughout the experimental section beginning on page 35.

To clarify this point, Applicants draw the Examiner's attention to Figure 4, which (as mentioned in its legend on page 33) illustrates the principle of the method according to the invention, applied to the detection of the V8-J recombinations. To this end, a single pair of primers (SEQ ID NO: 5, specific for the gene hTRAV8 and SEQ ID NO: 13, specific for hTRAJ41) is used, and as illustrated in the figure, eight different amplification products are simultaneously obtained, each corresponding to a different V-J rearrangement: rearrangement of hTRAV8 with hTRAJ41 leads to the smallest product (indicated "1"), rearrangement of hTRAV8 with hTRAJ42 leads to the second smallest product (indicated "2"), *etc.*, and rearrangement of hTRAV8 with hTRAJ48 leads to the biggest product which can be amplified in the amplification conditions used (indicated "8"). These data illustrate that, as stated in the paragraph on page 17, lines 10 to 23, the identity of the V and J genes which have rearranged in the amplified product can be determined by the primer V used (for the V gene) and by the size of the amplification product obtained with a given pair of primers (for the J gene).

Accordingly, Applicants believe that the amendments to the claims have addressed the basis for this rejection, and it is respectfully requested that the rejection be withdrawn.

Rejection under 35 USC § 103(a)

A. Claims 23-26, 28, 30, and 33-38 were rejected on page 8 of the Office Action as allegedly being obvious over Pasqual, as evidenced by Biochemica excerpt and Krangel, in view of GenBank GI:21363121 ("GenBank"), Wu, and Arstila.

Pasqual is an initial study of the ontogeny of the T cell repertoire in a murine model (see at least the last paragraph of the introduction, page 1164). Pasqual used a method of quantitatively evaluating V-J rearrangements of the TCRAD locus in mice, based on multiplex PCR. On page 11 of the Office Action, the Examiner notes several distinguishing features between the claimed invention and Pasqual. First, as described in the Materials and Methods of the article by Pasqual, genomic DNA was extracted from pools of thymic lobes from embryonic or neonatal mice (see page 1164, last sentence of the paragraph entitled "Mouse"). Hence, Pasqual performed an analysis of V-J alpha rearrangements on a sample which differed from the sample used in the claimed methods in that:

(i) It is of murine and not of human origin

(ii) Thymic lobes were extracted from mice which were necessarily euthanized; hence, the sample cannot be considered as a biopsy (nor as a blood sample)

(iii) Since the starting material used by Pasqual et al. is a pool of thymic lobes, the method disclosed in Pasqual et al. does not lead to the evaluation of V-J rearrangements in an individual, as claimed in claims 23 and 24.

Second, Pasqual fails to teach elongation steps lasting 10 minutes (Pasqual performed the elongation steps for 6 minutes). Third, Pasqual detected the amplified products by Southern blot, using radioactive probes. To the contrary, the methods according to the invention comprise a detection step directly on the gel, by use of a DNA labeling agent, for example a fluorescent DNA-labeling agent.

Applicants submit that a skilled artisan, reading Pasqual, could not have arrived at the claimed invention. Indeed, what appears from Pasqual is that the authors encountered

a significant sensitivity problem when performing their method, since they had to use a particularly sensitive method for detecting DNA products after their separation on a gel (*i.e.*, Southern blotting with a radio-labeled probe), despite the drawbacks of said detection method (see, *e.g.*, point 7 of the declaration under rule 132 by Nicolas Pasqual dated April 12, 2010): length, cost and risk. Moreover, even considering the benefit of a sensitive detection method, Pasqual still required the pooling of thymic samples from several sacrificed mice in order to obtain enough genomic DNA to perform the method (see page 1164, last sentence of the paragraph entitled "Mouse").

Based on this, Pasqual strongly taught away from the present invention, since the skilled artisan would never have contemplated that it could be possible to perform the method disclosed in this publication by using a less sensitive detection technique, starting from a smaller amount of genomic DNA. If one skilled in the art wanted to use a less sensitive detection method, they would have concluded that even more starting genomic material was required, not that less material could be utilized. The evident sensitivity problems of the method disclosed by Pasqual would have convinced the skilled artisan that this method was only available under very specific circumstances, when large amounts of genomic DNA from T cells are available. Such large amounts of genomic DNA from T cells are clearly not available when the starting material is from a biospy or blood sample from a single human individual.

The Examiner has considered that claims 23 and 24 were obvious to the combination of Pasqual, Arstila, and Barnes. However, Arstila and Barnes do not remedy the deficiencies of Pasqual for the following reasons: Arstila discloses a method for estimating the human T cell receptor diversity. The approach used in this article is based on the immunoscope methodology (see page 960, Note 4). For estimating the human T cell receptor diversity, Arstila analyzed the CDR3 region of a few V-J rearrangements, using as a starting template complementary DNA from peripheral blood T cells from a healthy donor (see page 958, column 2, lines 14-16). Arstila then performed PCR amplifications of fragments of less than 45 nucleotides (see, *e.g.*, Figure 1) to analyze the junctional diversity at a particular CDR3 (and not the combinatorial diversity of V-J rearrangements).

Applicants submit that Arstila cannot be combined succesfully with Pasqual for several reasons. First, it is respectfully submitted to the Examiner that in a T cell, the copy number of mRNA for a TCR is far greater than the copy number of genomic DNA encoding the same. Hence, despite the fact that reverse transcription is not always 100% efficient, the copy number of starting template is greater in the method disclosed by Arstila than in a biological sample in the sense of the present invention. Second, in complementary DNA from T cells, the proportion of DNA corresponding to the fragment to be amplified is far greater than in genomic DNA, since it is devoid of non-coding and non-expressed DNA. As an illustration of the discrepancy, only 1.5% of the human genome actually codes for proteins (and hence, is transcribed into mRNA), as evidenced by the enclosed Wikipedia article on Human Genome (see at least the second paragraph). Third, Applicants respectfully submit that amplifying a DNA segment of at most 45 nucleotides is easier and more efficient than amplifying long DNA fragments, as inherently required for performing the method disclosed in Pasqual. Therefore, Arstila would not have provided the skilled artisan with any motivation to transpose the fundamental research method disclosed by Pasqual to living humans. Indeed, nothing in Arstila suggests that the sensitivity problems encountered by Pasqual could be overcome when using human genomic DNA. Arstila merely provides the skilled artisan with an alternative method for estimating T cell receptor diversity in humans, said method being devoid of sensitivity problems.

The Examiner utuilizes Barnes for teaching PCR amplification of up to 35-kb DNA fragments, with extension steps performed at 68°C for 11-24 minutes, and direct visualization of the obtained products in gels run or later stained by ethidium bromide. Barnes, however, fails to overcome the deficiencies of Pasqual and Artsila to arrive at the claimed invention. Applicants respectfully submit that the skilled artisan could not have extrapolated the results disclosed by Barnes to the amplification and detection of large genomic DNA fragments from human biological samples. The template used by Barnes for amplifying large DNA fragments is lambda bacteriophage DNA (see title and first paragraph of page 2217). As evidenced by the enclosed Wikipedia article, the lambda bacteriophage genome is 48.5 kb long (see page 2, paragraph "Anatomy"). Since the size of haploid human genome is over 3 billion DNA base pairs (as evidenced at least by the

first paragraph of the enclosed Wikipedia article on Human Genome), the human genome is $0.7 * 10^5$ times bigger than the genome of lambda bacteriophage. Accordingly, to have the same number of copy of a given non-rearranged gene as used by Barnes, $0.7 * 10^5$ times more genomic DNA would be required, which means from 7 μg to 0.7 mg of genomic DNA. For rearranged genes, such as genes in the locus TCRAD in T cells, an even greater amount of genomic DNA is needed to have the same copy number as used by Barnes. An under-estimation of this amount is from 2 mg to 200 mg of genomic DNA. This presents two problems: (i) the PCR would be inhibited by the viscosity of the sample, due to the high concentration of DNA (see experimental data in Annex 1), and (ii) this amount of DNA cannot be obtained from a blood sample or a biopsy. This second point is discussed below.

As evidenced by the enclosed Wikipedia article concerning C-value, a unique diploid human cell comprises approximately 6.5 pg of DNA. Accordingly, $30 *10^7$ T cells would be required in order to obtain an underestimation of the number of template copies present in the lowest amount of bacteriophage DNA used by Barnes. This is clearly incompatible with the size of a sample according to the present invention, since a biopsy or a blood sample from a living individual can comprise, at most, 10^7 T cells.

Based on these differences, the teaching of Barnes would not have helped the skilled artisan overcome the sensitivity problem of the method described in Pasqual, since Barnes describes amplification of a long DNA fragment from a purified template of only 48.5 kb, which means that (i) Barnes performed the PCR amplification from a starting material comprising a copy number that cannot be obtained in a biological sample according to the present invention, and (ii) in the case of Barnes, a high copy number can be obtained without leading to an excessively high total DNA concentration, which would not be the case for human genomic DNA.

Due to the deficiencies and incompatibilities of the cited references, Applicants submit that the claimed invention is not obvious. The Office Action appears to have disregarded several limitations within each cited reference to allege that the claimed invention is obvious. However, taking the teachings of each as a whole, a skilled artisan could not arrive at the claimed invention. Only through impermissible hindsight does it appear that the significant limitations of each teaching appear to be irrelevant as the

inventors of the claimed invention have overcome them. As explained above, the cited references cannot be combined without acknowledging their inherent limitations. The court in *Motorola, Inc. v. Interdigital Technology Corp.*, 930 F. Supp 952, 974 (D.Del 1996) (aff'd in part & rev'd in part, 121 F.3d 1461, 43 USPQ2d 1481 (Fed. Cir. 1997)) stated the following concerning hindsight reproduction:

> A court may not, with 20-20 hindsight, utilize an inventor's claims as a template and reconstruct his invention willy-nilly by picking and choosing elements at will from the prior art.

Further, as stated in the 2010 Obviousness Guidelines from the U.S.P.T.O. (Fed. Reg. 75: 53643-53660, 2010 at 53658), non-obviousness can be shown when a person of ordinary skill in the art would not have reasonably predicted the claimed invention based on the prior art and the resulting invention would not have been expected. Thus, Applicants submit that the presently claimed invention is not obvious when the references are read in their entirety as required by law.

B. With regard to claims 25, 26, 28, 30, and 33-36, the Examiner has considered that claims 30, 35 and 36 were obvious with regard to the combination of Pasqual, Arstila, and Barnes. However, claim 30 is dependent upon claim 23 and claim 35 is dependent upon claim 24, which are inventive over the cited documents for the reasons discussed above. Hence, these claims are also not obvious over the combination of recited documents. Claim 36 has been cancelled without prejudice or disclaimer of its subject matter.

C. The Examiner has also considered that claim 25 was obvious having regard to the combination of Pasqual, Arstila, and Barnes, further in view of Wu. However, claim 25 is dependent upon claim 23, and Wu does not remedy the deficiencies of the combination of Pasqual, Arstila, and Barnes to render claim 23 obvious. Accordingly, claim 25 is not obvious over to the combination of the cited documents.

D. The Examiner also considered that claims 26 and 35 were obvious over the combination of Pasqual, Arstila, Barnes, and GenBank GI:21363121. However, these

claims are dependent upon claim 23, and GenBank GI:21363121 does not remedy the deficiencies of the combination of Pasqual, Arstila, and Barnes to render claim 23 obvious. Accordingly, claims 26 and 35 are not obvious over the cited documents.

E. Claim 27 has been rejected as allegedly being obvious over Pasqual. as evidenced by Biochemica excerpt and Krangel, in view of GenBank GI:21363121, Wu, Arstila, and Barnes, and further in view of GenBank 21536269.

Claim 27 is dependent upon claim 23. The reasons why claim 23 is not obvious over the combination of Pasqual et al., GenBank GI:21363121, Wu, Arstila, and Barnes have been discussed above. Applicants respectfully submit that GenBank 21536269 does not remedy the deficiencies of these publications, and therefore, claim 27 is not obvious over the cited documents.

F. Claim 29 has been as allegedly being obvious over Pasqual, in view of GenBank GI:21363121, Wu, Arstila, and Barnes, and further in view of Liljedahl and Perron.

Claim 29 is dependent upon claim 23. The reasons why claim 23 is not obvious over the combination of Pasqual, GenBank GI:21363121, Wu, Arstila, and Barnes have been discussed above. The only supposed additional teaching of Liljedahl and Perron is that the duration of the elongation steps can be incremented. These references therefore do not remedy the deficiencies identified here above. Claim 29 is therefore not obvious over the cited documents.

G. Claims 31 and 32 have been rejected as allegedly being obvious over Pasqual, in view of GenBank GI:21363121, Wu, Arstila, and Barnes, and further in view of Dau.

Claims 31 and 32 both pertain tomethods comprising a step of performing the method of claim 24. Claim 24 is not obvious over the combination of Pasqual, in view of GenBank GI:21363121, Wu, Arstila, and Barnes, for the reasons discussed above. The only additional teaching of Dau identified by the Examiner is the comparison of the T cell repertoire of a subject to that of a healthy human subject. Dau, therefore, does not

remedy the deficiencies identified here above. Accordingly, claims 31 and 32 are not obvious over the cited documents.

Conclusion

The foregoing amendments and remarks are being made to place the application in condition for allowance. Applicants respectfully request entry of the amendments, reconsideration and the timely allowance of the pending claims. A favorable action is awaited. Should the Examiner find that an interview would be helpful to further prosecution of this application, she is invited to telephone the undersigned at their convenience.

Respectfully submitted,
Morgan, Lewis & Bockius LLP

/Zachary Derbyshire/

Zachary E. Derbyshire
Registration No. 64,669

Dated: **January 11, 2011**
Morgan, Lewis & Bockius LLP
Customer No. **09629**
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
202-739-3000